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BLOOD-FEEDING BEHAVIOR OF ADULT *AEDES AEGYPTI* MOSQUITOES[1]

JACK COLVARD JONES[2] AND DANA RICHARD PILITT

Department of Entomology, University of Maryland, College Park, Maryland 20742

Of all the complex actions which mosquitoes perform in their lives, none are of such great interest to human beings as the blood-feeding behavior of the adult females. Except for the facts that the males do not pierce the skin to take blood and that the females readily do so, some of the simplest and most interesting features of blood-feeding have never been described or adequately studied, in spite of the fact that this act has been repeatedly observd since the first description by Reaumur (1738). The most detailed information on blood feeding in mosquitoes is given in Christophers' (1960) review. No one investigator has examined the general problem of blood-feeding behavior of any one species of mosquito so that our current knowledge is based on a series of isolated observations by many workers using different species. Since the work of Robinson (1939) on *Anopheles,* apparently no one has studied the effects of experimental surgery on the mechanism of fascicular insertion by mosquitoes. The present paper offers an overall picture of many aspects of blood feeding by known age individuals of a single species.

Methoods and Results

Bangkok and U. S. Naval Medical (U.S.N.M.) strains of the Yellow Fever mosquito, *Aedes* (*Stegomyia*) *aegypti* (Linnaeus), were reared in an insectary held at 27° C and 80% relative humidity. The adults were aged by noting the time when they emerged from the pupal case. The degree of engorgement on fluid was estimated qualitatively using the visual scale of Pilitt and Jones (1972). Unless specified differently, 10 individuals of each sex were employed in each test using a 1 cu ft cage.

Definitions

Since adult mosquitoes feed only on substances which can be drawn into their alimentary canals by suction, the term *feeding* will be defined here to include the uptake of any fluid into the alimentary system via the food canal, the latter being formed within an intricate arrangement of various fine feeding stylets (the fascicle) which are ensheathed by a large labium. The fascicle and the labium together comprise the conspicuous proboscis, at the anterior end of which are two sensory-haired labella.

The term *probing* has been used very loosely with regard to mosquitoes to refer to three very different acts: (1) a directional thrust of the whole proboscis

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without necessarily contacting a specific object, (2) the touching of the labella to the surface of an object, and (3) the insertion of a distal portion of either the fascicle or the proboscis into the test object. The following terms were devised to refer to each of these three kinds of probes: (1) *directional (proboscidal) thrust,* (2) *labellate* or *labellation,* and (3) *fascicular insertion* (where the fascicle alone is inserted) or *proboscidal insertion* (never seen during normal blood feeding).

Reactions of adult male mosquitoes to a human forearm

Exposing a bare human forearm within a 1 cu ft cage containing many resting adult male mosquitoes caused them to fly about almost at once. They made rapid flights along highly variable, usually widely elliptical, paths and often flew in figure-8 patterns. Some males would skim along about 3 inches or less from the arm and return frequently, generally parallel to it. Most of them landed on the hairs and skin. None of the males made any attempt to insert his proboscis or fascicle into the skin. When the tip of the labella did touch the skin, the maxillary palps were often raised. Individuals were observed to return repeatedly to the human forearm with or without labellating for periods up to $2\frac{1}{2}$ hrs, at which time the males appeared noticeably less agitated by the presence of the arm.

Open pool feeding on defibrinated sheep blood or a mixture of blood and sucrose

Males and females which had been fasted for 4 days were placed in a cage with a crucible containing whole sheep blood. While they did not all take blood, those which did so fed to a stage 3 or 4 on the engorgement scale. When the mosquitoes were offered a 1:1 mixture of sheep blood and sucrose, most of them rapidly visited the mixture and imbibed to a stage 4 on the engorgement scale. When males were offered a choice between 10% sucrose and defibrinated sheep blood, they quickly drank only the sucrose.

Initial reactions of adult female mosquitoes to human beings

Female mosquitoes were observed to approach clothed human beings in the laboratory. They almost never took a direct path when the distance between them and the human being was more than 3 ft. They often flew at a level below the waist in a very complex zigzagging path. Once within a range of 12 inches, they tended to fly in a rapid horizontal figure-8 pattern which would alternate with sudden up and down flights. They would rapidly retreat only to approach again. Most of the females landed on the ankles and legs and repeatedly thrust and labellated. Females also landed and fed on different regions of the head and neck.

Females often thrust their proboscides vigorously and repeatedly at a variable but rapid rate through clean mosquito netting to various extents. Such probing responses could generally be rapidly elicited by bringing the cage near a human arm or after breathing lightly on the netting. Females thus activated appeared to go into a probing frenzy, that is, they would make strong directional thrusts, labellate, and in some cases even attempt to pierce various portions of the cage in the immediate vicinity of a human being.

To determine the age at which females would take their first blood meal, 25

unfed mosquitoes of precisely known ages were exposed for 30 min periods to a bare human forearm daily for 4 to 5 hrs per day, from the time of their emergence from the pupal case until they were 4 days old.

When a hand was placed immediately in front of a very young female, no responses were elicited. The earliest that a female of the Bangkok strain was observed to take a blood meal was 21 hrs postemergence. The majority took their first blood meal when they were 23 to 24 hrs old. Some of the U.S.N.M. females were first observed to take a meal when they were 26 hrs old. In one test 3 out of 15 U.S.N.M. females never took a blood meal and died on the fourth day of the study.

Placing a human forearm in a cage of females which are ready to take their first blood meal, agitates some of them to fly. If they are less than 6 to 9 inches from the arm, they tend to land on it very quickly on any particular site. Typically, females land with 4 legs on the skin and either begin walking while making short directional thrusts or light labellar taps before settling on one site for puncture, or they begin almost at once to insert the fascicle at the initial landing site. Females will feed when their dorsal side is either up or down or faces laterally. While mosquitoes characteristically do not stand or feed with their heads facing downwards under normal conditions, it was discovered that if they landed on the arm and it was turned so that the mosquito's head and proboscis faced straight downwards, they could labellate, penetrate and fully engorge on blood. (Upon removing their fascicles, however, most of the mosquitoes somersaulted downwards due to the weight of the blood in their engorged abdomens, at which moment they took flight.)

Once a feeding site is determined, the females always lower their metathoracic legs before starting to puncture the skin. Shortly after the labella are firmly appressed, the maxillary palps are suddenly lifted to an angle of about 75°. Although one palp can be lifted with the other only partially raised, usually they are elevated simultaneously or nearly so. As soon as the fascicle begins to penetrate the skin, the palps begin to move up and down rapidly over a small angle of approximately 15°, and the head and antennae are seen to rock but at a slower rate than the palps. Often after a puncture has been made, a female will run the fascicle up and down within the wound. During puncture, a female's legs usually move slowly closer to her body, as the femoro-tibial angles becomes more acute. As penetration starts, the proximal portion of the labial part of the proboscis curves posteriorly in a hairpin fold, and the fascicle is then desheathed from the labium except at the labella. During penetration, the labium as a whole makes fine side-to-side movements. The fascicle is usually inserted into the skin for about one-half its length, although it may sometimes be pushed down close to the level of the distal end of the maxillary palps. The angle of the proboscis is 45° to 90° to the plane of the skin utilized. Rapid movements of the palps occurred until a bright red streak of blood suddenly appeared in the fascicle. As soon as this was seen, the palps abruptly stopped rocking. The sudden cessation of palpal movements indicates that imbibition has begun: the animal then becomes almost motionless, except for a small, intermittent, telescopic extension and retraction of the tip of the abdomen. When the female reaches a stage 5- on the engorgement scale, she expels the first droplet from her anus. Between stages 5- and 5 she continues to expel droplets. The first 1 or 2 drops

TABLE I

Duration of different phases of blood-feeding of the Bangkok strain of unrestrained Aedes aegypti *females*

Phase of blood feeding	Duration in seconds of different phases of feeding in			
	1 cu. ft. cage*		2 in. plastic cup**	
	Range	Mean and S. E.	Range	Mean and S. E.
Insertion of fascicle	16–105	53.3 ± 6.6	18–120	43.4 ± 5.1
Full engorgement (to stage 5)	103–225	155.3 ± 8.8	45–132	128.9 ± 8.4
Withdrawal of fascicle	2–4	5.4 ± 0.9	2–35	10.9 ± 1.7

* 14 mosquitoes which were 2 days old and fasted.
** 20 mosquitoes which were $3\frac{1}{2}$ days old and fasted.

appear either milky or clear, while subsequent ones are nearly always clear and of variable volumes. Engorged females after leaving the host were observed to expel droplets of clear fluid from the anus at a decreasing rate for a period up to 2 hrs or longer.

Withdrawal of the fascicle at the end of normal blood feeding was indicated by a renewed rocking movements of the maxillary palps, and these continued until the fascicle was fully removed. Fascicular withdrawal was either a simple direct steady removal or consisted of first withdrawing the fascicle and then reinserting it momentarily before total withdrawal. Some females (5%) were seen to reinsert their fascicles after a stage 5 engorgement had been attained and they continued to do this for as long as 1 min, occasionally making a series of reinsertions. During fascicular removal, a female straightens her prothoracic legs to lift her head and thorax, and then makes a backward angling movement with her whole body. During the act of fascicular withdrawal, the labium rocks from side-to-side as the distal end is slowly slid down the emerging fascicle. Once the fascicle has been removed from the skin, it springs upward and forward. Complete re-sheathing of the fascicle occurs after its complete withdrawal from the host. The final stages of re-sheathing consist of a short rapid back-and-forth pumping action of the labium as the fascicle is fitted back within its groove. Occasionally a female augments the final stages of re-sheathing by placing her front tibial spines in the area of the labial groove and slides them forward.

Duration of the three phases of the blood-feeding act

The duration of each of the three phases of the blood-feeding act (insertion of the fascicle, imbibition of blood, and withdrawal of the fascicle), as shown in Table I, was found to be highly variable, despite the uniformity of individuals and test conditions. When a forearm was offered to females held in small containers or in 1 cu ft cages, those in the small cages (2 inch plastic cups) required less time for insertion and engorgement. It can be seen for both groups that the insertion of the fascicle required much longer than for its withdrawal, while the duration of actual blood-intake was longer than other phases.

TABLE II

First and second blood meals of the U. S. Naval Medical strain of Aedes aegypti *females*

Individual number	Hours since first meal	Seconds			
		To locate arm and labellate		From fascicular insertion to withdrawal	
		first meal	second meal	first meal	second meal
1	2	82.0	46.0	176.0	201.0
2	2	7.9	35.7	202.9	158.7
3	2	44.4	28.6	315.0	205.0
4	3	89.6	32.4	374.6	165.7
5	5	58.9	32.8	352.5	130.3
Mean	3.0	56.6	35.1	284.2	172.1
S.E.	±0.6	±16.3	±3.5	±39.7	±14.9

The second blood meal

Immediately after taking a full blood meal, most females make no attempts to take another. A few females were seen to finish their first meal, run the tarsal spines down the labial groove repeatedly, and then reinsert the fascicle immediately afterwards, but without apparently taking any more blood, even though a streak of blood could be seen in the fascicle.

To determine how soon females would again take blood, they were offered the same forearm at hourly intervals for a 5 min period. As shown in Table II, each took another blood meal 2 to 5 hrs after the first feeding. The above results represent the maximum rate of feeding over a 5 hr period using a highly acceptable donor. The rate of refeeding was strikingly lower with another donor. From the time stage 5- on the engorgement scale is reached until the end of the blood meal and for several hrs thereafter, females excrete fluid, the loss of which allows for further imbibition. One can observe a similar refeeding by interrupting a female's initial blood meal at stage 4 + or 5-. Such females re-land and penetrate and imbibe within less than 1 hr. The variability of the time to locate the arm and the total feeding time for the second meal was strikingly less than for the first (Table II).

Feeding behavior following surgery

To determine some of the many parameters governing the process of blood feeding, a series of surgical operations were performed on females. In the first experiment, females which were previously seen to labellate were removed and anesthetized so that a small portion of the last tarsal segment could be removed from each leg. After recovery, many females tumbled or somersaulted upon landing on an exposed forearm. If they alighted on a flat plane or were tilted uphill, they could feed much more easily and rapidly than if the body was tilted downwards. A few females were seen engaging in a most remarkable act: they hovered

close to the arm, and while hovering, actually succeeded in just penetrating the skin. As soon as this happened, they landed and fed normally.

In the second experiment, one or more legs were cut off at the coxae. When only one or 2 legs were removed, feeding on a human forearm was not affected. When 3 legs were amputated, it appeared absolutely necessary for the remaining legs to form a tripod, with the legs being spaced further apart than normal to provide a stable base. The tip of the abdomen was generally lowered to serve as an additional support. After ipsilateral removal of 3 legs, some females placed one of their legs over to the legless side and took a blood meal. A female with 4 legs removed could still take a blood meal, provided that, of the remaining legs, one was in front and one was in the middle on the opposite side of her body. Penetration of the host by such a mosquito did not require a longer time, despite the relatively unstable base of the insect. The tip of the abdomen was used as a support from the time of landing until the mosquito flew away after taking a blood meal.

Various appendages of the head were removed. When the tip of the labella was cut off along with the tip of the fascicles, the mosquitoes would position their proboscides correctly but as their body weights were shifted in an attempt to penetrate the skin, the fascicles slid under their bodies.

When the fascicle was desheathed and only the lobes of the labella were removed, most females did not attempt to probe after landing on the arm. They would land and move the proboscis in small circles, but did not appress the exposed fascicle tips to the skin. A very few attempted to force the fascicle into the skin by pushing the whole body forward and downward. No desheathing or penetration was achieved, however, since the proboscis slid beneath the head.

When the fascicles were desheathed and their tips removed from a group of females, all landed on an exposed forearm but most did not probe. Penetration was not achieved by those which did probe, either due to a forward bending of the fascicle or inability to pierce the skin.

Two methods of artificial desheathing of the fascicle were used. Females desheathed by the MacGregor (1930) technique did not obtain a blood meal. Those attempting to do so, could not penetrate. Their proboscides repeatedly were pressed to the skin, but bent under the females' bodies. Using the loop method of temporary desheathing (Pilitt, 1971), 60% of the females tested were able to obtain a blood meal, but only after resheathing.

The effect of complete darkness on the ability of females to locate and obtain a blood meal was tested at 2200 hrs when the females had been in complete darkness for 2 hrs by inserting a forearm into the cage. All of the females took a blood meal during the 15 min test.

To study whether the antennae are critical to locating a human forearm, both of these sensory appendages were severed as close as possible to Johnston's organ from a group of females. Afterwards, a forearm was offered in a lighted insectary, at which time all of the females took a blood meal. The time required to locate the host was within the range of host-finding times of intact females. Another group of antennectomized females were offered a forearm in a completely darkened insectary at 2200 hrs and none of them took a blood meal within 15 mins.

Response of restrained females to a human forearm

Fasting females were etherized and glued to fine insect pins and after a 3 hr recovery were suspended over a forearm for 5 min, the arm being positioned to approximate an unrestrained feeding area. When the mosquitoes were held lightly against the forearm, some of them labellated and repeatedly attempted to penetrate, but the fascicles slipped off the surface of the skin to one side or the other, and none succeeded in penetrating the skin. When the females were placed on the arm at incorrect or awkward angles, they made no attempts to labellate. All of these non-labellating females had strongly upcurved proboscides after being placed in contact with the arm. It was obvious that even during the most careful artificial positioning, the angles of penetration did not begin to approximate the normal feeding stance of the unrestrained mosquito.

Responses of females to Galleria mellonella *larvae and* Hyalophora cecropia *adults*

Large last stage larvae of *Galleria mellonella,* which were reared at 35° C according to the method of Beck (1960), were placed in a 2-inch container into which fasting females were aspirated. During a 30-minute observation period, all of the mosquitoes approached and frequently landed and rode on the backs of the wriggling larvae and made directional thrusts, labellated, and repeatedly attempted to pierce the cuticle with their desheathed fascicle tips, usually at the intersegment. The mosquitoes were often prevented from penetrating the larvae due to the nearly constant writhings of the latter. One female which succeeded in piercing made only a very shallow insertion before being dislodged.

Larvae used in the preceding test were removed an immersed in a water bath held at 42° C for 5 mins, after which they were placed, without direct handling, on the floor of the cage containing the same group of females. Since the "coddled" larvae were not approached during a 5 min period, they were removed with forceps and lightly skewered with pins and attached to the sides of the screen near the top of the cage. All of the mosquitoes quickly landed on them, labellated, inserted their fascicles easily into the re-located larvae and most of them withdrew varying amounts of hemolymph from the hemocoele. While some females were seen to imbibe hemolymph which had oozed from the wounds made during skewering, most walked away and penetrated the cuticle some distance from the exposed hemolymph. Those females which had taken the most hemolymph were the ones which had re-visited and re-penetrated the larvae.

Restrained adults of both sexes refused to imbibe any hemolymph from freshly heat-fixed larvae when their proboscides were inserted into capillary tubing containing this fluid.

When 2 large adults of *Hyalophora cecropia* were placed in a cage with mosquitoes of both sexes, a few of each landed on the moths' wings and abdomens and both sexes made directional proboscidal thrusts, but the females were never seen to penetrate and obtain hemolymph.

Response of fasted females to recently-blood-engorged mosquitoes

Recently human-blood-fed mosquitoes were introduced into a 1 cu ft cage containing numerous unfed females, and none of the fasting individuals approached

the engorged ones during a 1 hr period at 27° C. When females which had just taken a human blood meal were aspirated into a much smaller cage (2 inch diameter) containing fasted females, none of the latter were obviously attracted to the blood-fed mosquitoes, but would occasionally bump into or walk over them and sometimes would rapidly labellate the walls of the cage or make frequent proboscidal thrusts through the screen flooring around them. When a fasted female touched a standing blood-fed individual, the latter would move slightly out of her path or would fly away.

When individual females, whose legs had been removed after blood feeding, were closely confined in very small compartments with unfed females, only 1 out of 20 of the unfed mosquitoes approached the immobilized one and penetrated the blood-distended abdomen and fed.

Since it had been reported that *Aedes aegypti* would rapidly feed on mosquitoes which had recently fed on chick blood (Wetthersby, Hyong-Sun, and McCall, 1971), it was decided to determine whether mosquitoes containing blood of a higher temperature than that of human beings would be more attractive to fasted females. When a week-old chick was held by hand in a cage of females, all of the mosquitoes quickly landed on the hand and none on the chick. When the chick was placed alone in the cage, the mosquitoes landed either on the feathered body or alighted near the eyes or on the legs where some obtained blood. As soon as they had engorged, they were introduced into a small container with fasted females. None of the latter approached or attempted to labellate or pierce the distended females.

The possible effects of higher temperature of imbibed blood on the attractiveness of fed females to fasted individuals were studied by immersing a group of human-blood–fed mosquitoes in a waterbath held at 42° C for 5 mins and quickly placing them in a small cage with fasting females.

During the first 7 mins of observation, the fasted mosquitoes indiscriminately labellated and made directional thrusts through the screening, and when they encountered blood–fed individuals, either avoided them or walked over their motionless bodies without labellating. After 7 mins 20 secs, a single fasted female directly approach and quickly began penetrating the pleura of one of the blood-engorged mosquitoes. Her fascicle was thrust deeply into the abdomen for almost its entire length, and blood could be seen passing from one abdomen to the next. This imbibing female repeatedly partially withdrew her fascicle and then re-inserted it in the same puncture site. This female was approached by two other fasted females which touched her body with their proboscides and immediately began to desheath their fascicles in an attempt to penetrate her abdomen. As they vigorously climbed onto or around her body, they violently probed with their glistening fascicular tips, her head, sides of thorax and abdomen, and especially her wings, but neither succeeded in penetrating. They did not labellate or thrust into the body of the heat-treated mosquito. The original imbiber continued to withdraw blood from the prone body of the heated female while kicking at the probing intruders.

The fascicle of one intruder thrust at the head of the imbibing female, slipped off onto the body of the heat-treated donor mosquito, and was inserted alongside that of the first imbiber, and withdrew blood from the site for a short time, before the displacement of the original imbiber. A third female now approached the heated mosquito and made a separate puncture and briefly imbibed blood and

withdrew her fascicle only to return and probe the wings and abdomen of the second imbiber without actually penetrating.

Many of the remaining fasting females now approached 3 previously ignored heat-treated mosquitoes 22 mins after being placed together. They repeatedly probed or made violent thrusts at different parts of them with their labella and fascicular tips. Probing activity seemed concentrated around the pleural regions of blood-filled abdomens. Attempts to penetrate them were almost continuous in spite of frequent failure to achieve insertion. Often during this frenzied activity, females were seen to probe others which were themselves in the act of penetrating the heat-fixed bodies. While no female successfully penetrated an active mosquito, many successfully punctured the pleura of the heated individuals and subsequently imbibed slowly from them with characteristically frequent partial withdrawals of their fascicles followed by re-penetrations. Often a second female which was actively thrusting would climb over and displace another feeding female and penetrate apparently the identical site. Those which used a new feeding site on the pleura often seemed to have some difficulty in penetrating the cuticle.

At the conclusion of the above observations, it was noted that the one heat-treated female which had been able to recover enough to walk about had not been penetrated, so that she remained full of blood. Only one of the previously fasted females which took blood was engorged, and the remaining imbibers took varying but generally small to moderate amounts.

Discussion

The present studies present a unified view of several major parameters involved in the blood-feeding behavior of a single species of mosquito. Although adult males of *A. aegypti* are clearly greatly attracted to man, their visits, though frequent, are of relatively short duration. While the males often lightly touch their labella to the surface of the skin and may momentarily closely appress them thereto, they were never observed to make any attempts to penetrate the skin. Males directionally thrust and labellate in a manner somewhat similar to the females, but, unlike the females, they lack maxillary stylets for cutting into the skin. Although a few dehydrated males will drink defibrinated blood from open drops, when given a choice between sucrose and blood, they choose the sucrose.

While it has been previously noticed that recently-emerged mosquitoes do not immediately take a human blood meal, broadly divergent data as to the time of her first blood meal are reported for *A. aegypti.* Thus, a range of 18 to 40 hrs is given by 4 different groups (Marchoux, Salimbeni and Simond, 1903; Peryassú, 1908; Mitchell, 1907; and Howard, Dyar and Knab, 1912). According to Macfie (1915), females feed for the first time on the second day, but more often on the third day. In the present work, the majority of the females took their first blood meal when they were 23 to 26 hrs old. Since newly emerged females are generally quiescent and not attracted to man until they are about 1 day old, it may be that host-seeking in females is activated by a hormone.

Although Gordon and Lumsden (1939) observed the raising of the maxillary palps in *Aedes* and recognized that this was the first sign of their intention to feed, they did not specifically correlate this with either insertion or withdrawal of the fascicle. In our study, activities of the palps were found to be directly correlated

with specific phases of the feeding act. Thus, raising of the palps occurred whenever the labella were appressed to an acceptable substrate. Initiation of palpal rocking was found to indicate the beginning of maxillary stylet activity associated with fascicular penetration. The sudden cessation of palpal movements signals the presence of blood in the fascicle. The resumption of movement marks the beginning of fascicular withdrawal. Utilizing this information has permitted, for the first time, a truly accurate recording of the times involved in the various phases of blood-feeding (penetration, imbibition and withdrawal).

The excretion of droplets during and at the end of blood-feeding has been observed by Bonne-Wepster and Brug (1932) and Christophers (1960). Although Boorman (1960) observed anal discharges 5 to 15 mins after withdrawal of the fascicle, we have seen excretion occuring over a 2 hr period and probably for much longer, but at an ever-slowing rate.

Robinson (1939) thought that *Anopheles* females required a thrust of the whole body in piercing the human skin, and Christophers (1960) theorized that the legs provide a fulcrum for this action. The present work provides the first direct evidence supporting this theory. The necessity of a fulcrum as opposed to a strong thrust was shown by the severing of various combinations of legs: in all cases, some type of support near the front of the thorax was necessary, whether provided by a remaining prothoracic leg or a mesothoracic leg which was placed further forward. While *A. aegypti* do not make obvious or vigorous forward thrusts at the moment of fascicular insertion, their bodies are inclined forward and downward during penetration. When all of the tarsi were removed so that the height of the mosquito was lowered significantly (presumably this lowering would thereby decrease the power of a leg-initiated thrust), they were all able to pierce the skin and take blood rapidly. However, the loss of either the tarsal tips or the entire tarsi of all the legs reduced the number of their feeding sites and generally forced them to use those flat or slightly inclined areas which did not require grasping. It should be noted, however, that in a few cases a female was able to penetrate a vertical surface of a forearm while hovering over the area and that only after initial fascicular penetration had occurred were all the legs placed on the host and wing activity ceased. Thus, it has been shown that any combination of legs or positioning thereof sufficient to maintain the proboscis at a suitable height above the substrate prior to penetration, without excess pressure on its tip, and sufficient to allow subsequently a slight controlled force to be applied to the fascicle during the penetration period could explain this phenomenon. It is possible, but unproven, that the teeth on the maxillary stylets pull the fascicle into the skin as opposed to being pushed by the body, and thus tilting of the body of the mosquito may merely represent the resultant accomodation to the lowering of the head. The weight of the mosquito's body alone, of course, may exert sufficient force upon the fascicle. The removal of the tarsi presumably decreases the forward thrusting ability of the mosquito, but would not significantly affect the force due to a shift in the position of the body. Thus, it would seem that a fulcrum rather than a thrust is the probable mechanism involved in skin penetration.

Harris and Cooke (1969) stated that *A. aegypti* would land and feed on living *Galleria* larvae, and they suggested that mosquitoes might be able to obtain enough protein from the hemolymph of some insects in nature to develop a few eggs.

While some individuals of our strains rapidly landed on active *Galleria* and vigorously attempted to penetrate them, only one out of 5 succeeded in doing so only momentarily and was dislodged before she could withdraw hemolymph. It appeared that the general failure to achieve penetration was due to the vigorous writhings of the larvae. Harris and Cooke (1969) also found that when *Galleria* had been "coddled" and suspended from the sides of a cage, female mosquitoes landed on them and fed. In the present work, it was observed that if the heat-treated larvae were not suspended, the mosquitoes did not land on them. The probable reason for this may be that specimens of *Galleria* are not very attractive to begin with and were discovered only because they were placed where the mosquitoes frequently landed, that is to say, a mosquito normally lands and rests on the vertical sides of a cage or on the roof and generally does not land or walk on the floor. Suspended heat-treated *Galleria* attracted many females which readily penetrated and withdrew varying amounts of hemolymph. They imbibed more slowly than with human blood. While Harris, Riordan and Cooke (1969) showed a fully engorged *A. aegypti* female still inserted into a *Celerio euphorbiae* larva, our mosquitoes never fully engorged on *Galleria*. Although Harris and Cooke (1969) stated that their *Galleria*-fed mosquitoes were able to lay eggs, our experiences indicate that hemolymph feeding is not an adequate substitute for vertebrate blood.

Weathersby, Hyong-Sun and McCall (1971) discovered that fasting *A. aegypti* were rapidly attracted to female mosquitoes which had just taken a blood meal from a chick. They also noted that the fasting females easily penetrated and withdrew blood from apparently unresisting engorged mosquitoes. Our strains of *A. aegypti* when fed on human blood were definitely not attractive to fasted mosquitoes either in a 1 cu ft cage or in small plastic containers, even under crowded conditions immediately following a blood meal on either a human or a chick. It was further observed that when the engorged females were touched, apparently by chance, by a fasting female, they moved away. Even after removing the legs of human-blood-fed females, they still did not attract fasting females which were relatively close to them and they were not penetrated, even if encountered, unless both the engorged and fasted mosquitoes were very closely confined. However, it was found that if the human-blood–fed females were heated to 42° C prior to being offered to fasting females in a 2 inch cage, they were attractive, but not immediately after being treated. Since it took at least 7 mins before the first apparently directed visit occurred, it is difficult to believe that the blood within the engorged mosquito would have retained its additional heat this long. A probing frenzy elicited in fasting females by the near presence of such heat-treated bodies of the engorged mosquitoes has never been described. The cause of this frenzy remains to be determined.

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Summary

1. General reactions of adult male and female *Aedes aegypti* to human beings in the laboratory are briefly described.

2. Males fly about for a long time in the presence of a human forearm in a cage, and they frequently land and walk about briefly on the skin. Although they touch their labella to the skin, they were seen to attempt to penetrate it.

3. When males were offered a choice between pools of sucrose and defibrinated sheep blood, they drank sucrose.

4. Most adult females take their first blood meal when they are 23 to 26 hrs old. As soon as a feeding site has been located, they lower their hind legs.

5. The behavior of the maxillary palps indicates the major phases of the normal blood-feeding act. When an acceptable substrate is encountered, the palps are suddenly raised. When penetration of the skin starts, the palps begin to move up and down rapidly. As soon as blood appears in the food canal, the palps abruptly stop moving. They begin to move again as soon as the fascicle begins to be withdrawn, and they continue to move until the fascicle is completely removed from the wound.

6. Females always insert their fascicles more slowly than they withdraw them. Engorement on blood varied from 45 to 225 secs. After taking a full blood meal, females will seek and rapidly take another 2 to 5 hrs after the first.

7. Before becoming fully engorged on blood, a female begins to excrete fluid from her anus and continues to do so for 2 hours after completing the meal.

8. Various surgical operations showed that females could take blood when all of their tarsal tips were removed and when all but two of their legs had been amputated. They could not penetrate the skin after their labella were cut off or when the labial sheath was removed, even though they could position themselves correctly on the skin and attempted to pierce.

9. Although females with both antennae removed can locate and feed upon a human forearm in a lighted insectary, they cannot do so in total darkness.

10. Females which had been glued to pins and oriented carefully on a human arm did not penetrate or take blood, probably because the angle for penetration is highly critical and was not approximated.

11. *Aedes* will land on and probe active *Galleria* larvae and *Hyalophora* adults but do not obtain hemolymph from them. Although females can imbibe hemolymph from heat-fixed *Galleria* larvae, they actively reject heat-fixed hemolymph when their proboscides were forced into it.

12. Fasting *Aedes* females did not approach or attempt to probe normally resting or immobilized adults which had just engorged on either a young chick or a human being, except for one case involving very close confinement. Some fasting females were observed to take varying amounts of blood from immobile adults which had been heated to 42° C, but only after a lapse of 7 to 22 mins.

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